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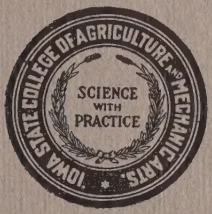

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A STUDY OF THE CONTROL OF CROWN GALL ON APPLE GRAFTS IN THE NURSERY*

BY I. E. MELHUS AND T. J. MANEY

Altho crown gall is one of the most serious diseases of apple trees in the nursery, effective control measures for root-grafted stock have not yet been found. This is doubtless due to previous lack of definite knowledge of the causal agent. Now, due to the excellent work of Erwin F. Smith and C. D. Townsend (7), *Bacterium tumefaciens*, Sm. and Tw., has been shown to cause this disease. Later, Smith, Brown and Townsend (6) contributed many other important facts pertaining to the pathogenicity and biology of the organism. As a result of these studies, experiments on control measures have been made possible and their successful conclusion has been made probable.

The pathogenicity of the organism causing crown gall is such that curative means are quite useless, altho several obviously futile attempts have been made along this line. The most logical means of attacking the problem of the control of crown gall is thru prevention, not cure, and in the case of grafted apple stock, prevention must begin with the apple graft.

In a recent bulletin, Greene and Melhus (1) have shown the undesirability of setting crown-gall-infested trees in an orchard. It also was made clear that trees galled at the union or on the stock made the poorest growth. Hedgecock (2) and others have shown that crown gall develops most commonly at the union. This is doubtless due to the fact that apple grafts, when set, carry a wound filled with meristematic tissue at the union, coupled with the fact that the crown gall organism flourishes best in this type of tissue. This would strongly suggest that control measures should be directed so as to prevent the plant pathogen from entering the wound.

PREVALENCE AND DESTRUCTIVENESS

As brought out by Hedgecock (2) and Smith, Brown and Townsend (6), crown gall is apparently general in its distribution in nurseries over the United States. The condition in Iowa is no exception. The disease occurs on a wide range of roseaceous

*Acknowledgments are gratefully made to Laurenz Greene, Purdue University, Lafayette, Indiana. Mr. Greene was formerly chief of the Pomology Section of the Iowa Agricultural Experiment Station, and until 1916 was actively connected with the planning and direction of the experiments described in this bulletin.

E. S. Welch, Mt. Arbor Nursery, Shenandoah, Iowa, generously donated the land, stock and labor with which to carry on the work of the project.

nursery stock and causes the elimination of a considerable percentage of the stock intended for the market.

Some idea of the prevalence of crown gall under nursery conditions can be gained from the checks in our experimental plots on five different varieties, namely: Wealthy, Patten Greening, Benoni, Jonathan and Wolf River. These trees were scattered in lots of 50 to 1,000 in four rows about 120 rods long. The figures are given in table I. The amount of crown gall ranged

TABLE I—THE AMOUNT OF CROWN GALL ON APPLE GRAFTS PLANTS IN 1916 AND DUG IN 1919

Exp. No.	Variety	No. grafts set	Percentage stand 1919	Percentage gall 1919
1-1-2	Wealthy	350	69.4	35.4
N-5	Wealthy	100	69.0	10.2
H-7	Wealthy	50	90.0	20.0
G-7-5	Wealthy	1000	65.9	26.9
G-3	Wealthy	500	60.6	22.8
G-7	Wealthy	2000	65.4	20.9
F-2-2	Wealthy	500	73.3	19.6
H-1	Wealthy	50	66.0	37.5
TOTAL		4550		
P-8	Patten	300	47.0	27.7
0-15	Patten	200	68.5	30.7
TOTAL		500		
1-3-2	Benoni	200	67.0	23.3
J-4-2	Jonathan	200	26.0	4.0
	Wolf River	200	63.6	14.6

from 4 to 37.5 percent, with a mean in the Wealthy of 19.2 percent, Patten Greening 29.2, Benoni 23.3, Wolf River 14.6, and Jonathan 4 percent. The difference in the amount of crown gall on the different varieties may be due in part to the limited number of trees in some of the experiments, or possibly in some cases to differences in degree of resistance.

Field observations, made at the time apple trees were being dug in the nursery, showed that a large number of trees were being discarded because of crown gall. Knowing the percentage of trees that develop crown gall each year, the financial loss can be calculated. The normal stand in a block of Wealthy trees consisting of about five acres, planted in 1916, as determined by counts, showed that it was 57.2 percent. The stand in the check lots in the field trials, recorded in this paper, was evidently somewhat better than the general condition in the field. About 20,000 grafts were set per acre, giving a 57.2 percent stand, or 11,440 trees of all grades. If 19.24 percent, as shown by the counts, are infected with crown gall, each acre produces 2,195 unsalable trees due to crown gall alone. At the present prices of apple trees this entails a heavy financial loss.

INFECTION IN RELATION TO THE CONDITION OF THE HOST

With the object of preventing, wholly or in part, such losses as have just been described, two types of studies were undertaken:

1. Infection and susceptibility of host tissues under diverse conditions.
2. The influence of disinfectants and fungicides in preventing the development of crown gall.

In the preliminary studies of infection, either viable organisms were applied to apple grafts just as they were set, or the grafts were set in a plot that was positively known to be heavily infected. The susceptability of the stock material was determined by planting seedling roots without previously making them into grafts. Subsequently, such questions as the influence of the stage of callousing, different methods of making grafts, influence of storage, and transmissibility of crown gall infection from stock to scion, were studied.

In the infection studies, *Bacterium tumefaciens* was grown in bouillon cultures and applied to the grafts, as described in table II. The grafts were grown for one year in a soil that was being used for growing a crown-gall-infected orchard. Notes were taken as to the growth of the trees and percentage of crown gall when they were dug. Altho the lots of grafts were small, ranging ing from 33 to 51, and grown only one year, yet the percentage of crown gall was so high that there can be no doubt as to where, and how quickly, infection takes place. When the organism was applied by dipping the trees into the bouillon cultures, every tree became infected.

The two check lots showed 82 and 100 percent infected, respectively. The grafts that had the bark injured, due to rough handling and hacking with a knife, also developed a high percentage of crown gall. Where the grafting knife was dipped in bouillon cultures while the workman was making the grafts, the percentage of crown gall was only 71.4 percent. The limited number of grafts used may have accounted for this discrepancy.

Probably the most instructive evidence obtained in these studies was the response, after planting, of the French crab roots when cut to lengths corresponding to grafts. Only 20 percent

TABLE II—INFECTION EXPERIMENTS ON APPLE GRAFTS WITH *BACTERIUM TUMEFACIENS*

Variety	Treatment	No. grafts set	Percentage stand	Growth	Percentage galled
Patten	Grafts dipped in dilute bouillon culture of *B. tumefaciens*	51	88.2	Medium	100.0
Patten	Sprayed grafts with bouillon culture of *B. tumefaciens*	33	90.9	Medium	83.3
Patten	Check	49	89.0	Medium	78.5
Wolf River	Knife dipped in culture of *B. tumefaciens* when making grafts	49	100.00	Good	71.4
Wolf River	Grafts with bark of stock and scion badly injured	44	100.00	Very good	100.0
Wolf River	Check	47	91.5	Poor	100.0
French Seedlings	Roots cut to lengths equal to grafts	49	93.8	Medium	20.0

became infected and the galls were all on the basal cut surface of the root. This suggests that the stock, even when injured, is not as susceptible as the scion wood.

INFLUENCE OF THE DEGREE OF CALLOUSING ON THE DEVELOPMENT OF CROWN GALL

It was felt that the extent of callousing might influence the susceptibility of grafts to crown gall. By holding the apple grafts under different conditions of moisture and temperature after they were made up, the callous formed varied materially at planting time. In some cases, the callous had scarcely begun to form, while in others it was quite advanced and the stock and scion were well united. As shown in table III, 5000 trees, divided into six lots, were used in this experiment. They were planted in the spring of 1916 and dug in the fall of 1919. In them, as in most of the experiments, the routine work of planting and subsequent cultivation was done by employees of the Mt. Arbor Nursery Company.

The stand varied from 60.6 to 75.4 percent. In all of the experiments except one, the percentage of crown gall ranged from 20 to 26.9 percent. The extent of callousing when the grafts are set seems to have little or no bearing on the development of crown gall, according to the data in hand. It would seem that grafts that have calloused but little when set would show the greatest amount of infection. In order to answer this question definitely, one would need either several years results under field conditions, or controlled experiments under laboratory conditions. The one-year results reported here serve merely as an indicator.

METHOD OF MAKING GRAFTS AND CROWN GALL DEVELOPMENT

Nursery men contend that the method of making grafts has an important bearing on the amount of crown gall infection, or in other words, well-made grafts are less liable to be infected than

TABLE III—DEGREE OF CALLOUSING AND CROWN GALL DEVELOPMENT
Wealthy

Expt. No.	Storage Conditions				No. grafts	Percentage stand		Percentage galled
	Packing material	Temperature	Time storage	Degree of callousing	set	1917	1918	1919
G-1	Moss and sand	40° F.	2 mo.	Normal	500	75.6	73.2	20.0
G-2	Moss and sand	40° F.	3 mo.	Excessive	500	79.0	75.4	25.5
G-3	Moss and sand	32° F.	2 mo.	Slight	500	63.8	60.6	22.8
G-4	Moss and sand	40° F.	2 mo.	Excessive	500	71.0	67.0	No data
G-7-5	Moss and sand	40° F.	5 days	Very slight	1000	69.0	65.9	26.9
G-5	Shingle shavings	40° F.	3 mo.	Excessive	2000	70.6	65.4	20.9

TABLE IV—METHOD OF MAKING APPLE GRAFTS AND CROWN GALL INFECTION

Exp. No.	Variety	Treatments	No. grafts set	Percentage stand			Percentage galled
				1917	1918	1919	1919
H-1	Wealthy	Perfect fitting grafts	50	66.6	66.0	64.0	37.5
H-3	Wealthy	Smaller root than scion—exposed surface	50	84.0	80.0	80.0	25.0
H-4	Wealthy	Rough graft	50	68.6	68.0	68.0	44.1
H-5	Wealthy	Split scion	50	84.0	82.0	82.0	39.0
H-6	Wealthy	Snout over base of scion	50	56.0	56.0	32.0	25.0
H-7	Wealthy	Cut surface of root exposed	50	90.0	90.0	90.0	20.0

poorly made grafts. The data in table IV, altho based on a comparatively small number of grafts, indicates that the method of grafting is perhaps not as important as has been previously considered. It is desirable to produce well made grafts in which the stock and scion fit perfectly, but in this the nurseryman is dependent upon the experience of the man making the grafts.

In table IV, it will be noticed that the percentage of gall on H-1, perfect fitting grafts, is greater than on H-6 and H-7, the most poorly made grafts in the experiment. It is quite probable that if other conditions are equal, well made grafts will show the least gall. However, since both types of grafts carry open wounds, the possibility of infection is dependent more on the presence of viable organisms than on any other single factor. That a wound materially facilitates infection is forcefully shown in the following studies, where the union was covered with a cloth.

THE EFFECT OF CLOTH WRAP ON THE DEVELOPMENT OF CROWN GALL

Hedgecock (2) showed that cloth used as a wrap on apple grafts materially reduced the percentage of crown gall, but he makes no reference to an injurious effect due to such a wrap.

In 1916 the effect of calico and cheesecloth, with and without the usual string wrap, plus bordeaux mixture, was studied. However, only 50 trees were used and these were allowed to stand for one season only. The results alone are, therefore, not conclusive, but strikingly confirm Hedgecock's findings, and suggest that the union which is wound with string is the chief point of attack on the part of the bacteria.

As shown in table V, little crown gall developed where the unions were well protected with cloth and bordeaux mixture, or cloth alone, as shown in experiment in P-6. In experiment P-7, where the grafts were rewrapped with No. 20 cotton string over the original string wrap, and treated with bordeaux mixture (25-25-50), 35 percent of the trees became galled. This condition was brought about by the heavy wrap of string girdling the stem and inducing excessive proliferation of the tissues. In experiment P-9, where the grafts were set after removing the

TABLE V—THE VALUE OF CLOTH WRAP IN PREVENTING CROWN GALL

Exp. No.	Variety	Treatments	No. grafts set	Percentage stand		Percentage galled
				1917	1919	1919
P-5	Patten	B. M. (25-25-50) calico over string	500	41.0	38.0	3.0
P-6	Patten	Check ordinary string	200	40.0	28.0	2.2
P-7	Patten	B.M. (25-25-50) double wrap of string	700	30.5	23.1	35.0
P-8	Patten	Check ordinary string	300	46.6	45.3	31.4
P-9	Patten	Check, no wrap; untreated	200	50.0	45.0	11.4

original string wrap, only 11.4 percent of the trees developed crown gall. This is a strikingly low percentage when compared with experiment P-8, a check, where 31.4 percent of the trees became infected. At the present writing this irregularity is not understood. However, it is clear, as was brought out by Hedgecock, that cloth wrapped about the union tends to prevent crown gall infection, but a cloth wrap at present is impractical from an economical standpoint in the production of apple grafts. The use of waxed paper instead of No. 20 cotton string was not tried in these experiments, but observations show that it loosens and falls away from the graft too soon after it is set to have any influence on the prevention of crown gall infection.

TRANSMISSIBILITY OF HAIRY ROOT FROM STOCK TO SCION

The question is often asked whether or not seedling trees that show some hairy root can be safely used for making apple grafts. Evidence brought out in table VI shows that hairy root infected seedlings remain infected when used as stocks and that treatment does not control the infection, as shown in experiment J-1-1. Where Wolf River seedling stocks, infected with hairy root, were treated with bordeaux 25-25-50, they showed only 7.7 percent crown gall. The Jonathan grafts treated carry out the same relationship as that shown in the Wolf River treatments, even tho the Jonathan is less susceptible. Scion wood grafted on hairy root stock does not develop galls or hairy root infection above the union to any greater extent than scion wood grafted on healthy roots. The infection in the stock tends to remain localized. All seedlings having crown-galled or hairy roots should, without question, be discarded.

TABLE VI—TRANSMISSIBILITY OF HAIRY ROOT

Exp. No.	Variety	Treatments	No. grafts set	Percentage stand		Percentage galled
				1917	1919	1919
J-1-1	Wolf River	B.M.(25-25-50)on hairy root seedlings	200	53.5	47.0	50.0
J-1-2	Wolf River	Check on hairy root	300	48.6	40.2	30.6
J-2-1	Wolf River	B. M. (25-25-50) on No. 1 roots	200	45.0	42.5	7.7
J-2-2	Wolf River	Check on No. 1 roots	300	71.6	63.6	21.9
J-3-1	Jonathan	B. M. (25-25-50) on hairy root	200	74.0	60.5	37.8
J-3-2	Jonathan	Check on hairy root	300	81.6	57.3	35.9
J-4-1	Jonathan	B. M. (25-25-50) No. 1 roots	200	22.0	10.5	25.0
J-4-2	Jonathan	Check on No. 1 roots	200	26.0	26.0	4.0

SCION WOOD FROM CROWN GALL INFECTED TREES

Some nurserymen cut scion wood from trees infected with crown gall on the underground parts, because such trees have no commercial value. This raises the question of whether or not this is a dangerous practice. Is such wood more likely to be internally or externally infected than wood taken from healthy trees? In other words, does the crown gall organism show internal bacterial strands in the apple, as described by Erwin F. Smith (5) for the Paris Daisy?

In order to obtain some data on this question, the experiments shown in table VII were carried out. Wood was cut from both healthy and badly galled trees and made up into grafts. Two varieties that are quite susceptible to crown gall were used, namely: Wealthy and Benoni. Grafts were made from scion wood from healthy and diseased trees, and a part of each lot was treated with bordeaux mixture (25-25-50), the remainder being left untreated as a check. In the case of the Wealthy grafts in the lot made from healthy wood, the treated showed 18.3 percent galled trees after two years, while the treated lot from diseased wood showed 15.2 percent. The checks showed 35.4 and 29.4 percent galled trees, respectively. The data with this variety indicates that the amount of crown gall was not increased when the scion wood was cut from diseased trees. The data on the variety Benoni supports that shown for the Wealthy variety, healthy wood treated gave 11.7 percent gall, diseased wood treated, 14.0 percent; while the untreated lots gave 23.3 and 32.4 percent gall on the healthy and diseased wood, respectively. Considering the response obtained with these two varieties, there is no consistent evidence supporting the contention that wood from infected trees will produce a higher percentage of crown gall.

Further study was made of the question of internal infection of scion wood by galls on trees showing infection below the soil level. This study was made in the greenhouse in the following manner: three-year-old Wealthy trees infected with crown gall

TABLE VII—A COMPARISON OF AMOUNT OF CROWN GALL DEVELOPING ON GRAFTS MADE FROM SCION WOOD CUT FROM HEALTHY AND DISEASED TREES

No. Exp.	Variety	Treatments	No. grafts set	Percentage stand		Percentage galled
				1917	1919	1919
1-1-1	Wealthy	B.M.(25-25-50)scion wood from healthy trees	300	39.3	36.3	18.3
1-1-2	Wealthy	Check scion wood from healthy trees	350	70.0	69.4	35.4
1-2-1	Wealthy	B.M.(25-25-50)scion wood from diseased trees	300	55.6	54.3	15.2
1-2-2	Wealthy	Check scion wood from diseased trees	300	61.3	54.6	29.4
1-3-1	Benoni	B.M.(25-25-50)scion wood from healthy trees	100	41.0	38.0	11.7
1-3-2	Benoni	Check scion wood from healthy trees	200	68.5	67.0	23.3
1-4-1	Benoni	B.M.(25-25-50)scion wood from diseased trees	200	51.0	50.0	14.0
1-4-2	Benoni	Check scion wood from diseased trees	300	75.0	70.3	32.4

were cut off from two to six inches above the gall before being set in 10-inch pots; the purpose being to induce the plants to send up new, soft, rapidly-growing shoots, into which the crown gall organism might travel. Twenty-five trees received this treatment in the spring of 1916 and were permitted to grow until fall. No signs of galls occurred on the new wood. In other trees, the shoots were removed and isolations made within two inches of the initial gall. No organism was found where outside sources of contamination were excluded. Not enough work has been done along this line as yet to permit definite statements, but it is questionable if internal infection of scion wood occurs.

There are, however, two other significant facts revealed in these data; first, the reduction in stand of the apple trees and, second, the reduction of crown gall in the treated lots. The treatment with bordeaux mixture (25-25-50) reduced the stand in the grafts made from healthy wood of the Wealthy variety from 69.4 to 36.3 percent; in the Benoni, from 67 to 38 percent. The percent of crown gall, however, was reduced in the grafts from healthy wood; in the Wealthy, from 35.4 to 18.3 percent; in the Benoni, from 23.3 to 11.7 percent. In the grafts from diseased wood, the reduction was again marked, in Wealthy from 29.4 to 15.2 percent and Benoni from 32.4 to 14 percent. The toxic effect and germicidal value of bordeaux mixture will be discussed in a later chapter.

THE INFLUENCE OF DISINFECTANTS AND FUNGICIDES ON APPLE GRAFTS AND CROWN GALL

The studies in the previous pages suggest that local infection is induced by *Bacterium tumefaciens* entering the tissues of the apple graft from the surface. This led to the study of the effect of certain disinfectants and fungicides on the grafts before planting. Considering that the grafts are building up meristem tissue at the union, as well as on all other cut surfaces, it was decided to study the effect of surface disinfection on the host, together with the prevention of infection by *B. tumefaciens*.

EFFECT OF SURFACE DISINFECTION ON APPLE GRAFTS

The common disinfectants, formaldehyde, mercuric chloride, and copper sulfate, were used. Apple grafts were dipped in solutions of different concentrations and planted in the greenhouse under conditions comparable to those existing in the field. It was found that the above disinfectants were injurious to callousing at the following dilutions: 0.16 percent formaldehyde, 0.25 percent copper sulfate, and 0.1 percent mercuric chloride. This suggested that grafts were readily injured by poisonous soluble substances which came in contact with the union and

TABLE VIII—EFFECT OF SURFACE DISINFECTION ON APPLE GRAFTS

Exp. No.	Variety	Treatment	Fungicide	No. grafts set	Pct. stand	Pct. galled
8	Wealthy	Grafts made and stored 2½ months	Lime sulfur (1-10) 30 seconds	49	79.6	100.0
9	Wealthy	Grafts made and stored 2½ months	Formaldehyde (1-240) 30 seconds	46	45.6	66.6
10	Wealthy	Grafts made and stored 2½ months	Mercuric chloride (1-1000) 30 seconds	48	66.6	80.0
11	Wealthy	Grafts made and stored 2½ months	Copper Sulfate (1-250) 30 seconds	47	42.5	50.0
12	Wealthy	Grafts made and stored 2¼ months	Check	50	68.0	83.3
20	Wolf River	Newly made grafts	Mercuric cl. (1-1000) 30 seconds	46	0.0	0.0
21	Wolf River	Newly made grafts	Check	48	85.4	77.7

that a soluble fungicide would not lend itself to the practice of preventive measures as well as one less soluble.

Further evidence supporting this contention was secured under field conditions in 1916, when small lots of grafts were dipped just before planting, as shown in table VIII, and planted in crown-gall-infected soil. In these trials, lime sulfur was added. The trees were dug after one year's growth. In the fall, the number of living trees and percentage of infection was determined. All of these disinfectants reduced the stand, as compared with the checks. The surface disinfection did not to any marked extent prevent the amount of crown gall. It is worth while to call special attention to the inefficiency of lime sulfur, which was used at a dilution of one part in ten of water. Here, as in the senior author's (3) studies of the toxic action of lime sulfur on germination of spores of *Phytophtora infestans* and *Plasmopora viticola,* it proved but slightly toxic as compared to copper sulfate.

THE EFFECT OF SURFACE DISINFECTION ON THE SUBSEQUENT DEVELOPMENT OF CROWN GALL

A study was made, not only of the effect of surface disinfection of the grafts, but also of the effect on crown gall infection. Lots of 100 trees each were dipped in formaldehyde (1-240), copper sulfate (1-16), and mercuric chloride (1-1000) for 15 minutes just before planting and allowed to grow three years under regular nursery conditions. A lot of 100 trees was held as a check. The stand at the end of the third year was 28, 0, 28 and 69 percent. The percentage of trees showing crown gall in these trials was 10, 0, 10.7 and 10.2, respectively. It is to be noted that the check showed over twice as many living trees and no more crown gall than the dipped lots. Again, it is obvious that dipping grafts in dilute disinfectants injured the grafts and did not check the development of crown gall.

In this same series of experiments, 100 grafts were dipped for 15 minutes in water suspension of lead arsenate (1-133). Here the stand was nearly equal to the check, 63 percent, and the

amount of crown gall was 3 percent less than in the check. The significant point in this test was not the reduction in the amount of crown gall, but the lack of serious injury to the stand with the lead arsenate. This lack was probably due to its solubility and toxic action. The precipitate adhered to the string wrapper on the union and went into solution slowly thruout the first growing season. Grafts that had been dipped in lead arsenate have been taken up in the fall and evidence of the precipitate found still present on the bark and string wrap. This suggested experiments with a fungicide known to be slowly soluble, namely, bordeaux mixture. The precipitate of bordeaux mixture is surprisingly insoluble; 4 CuO. SO^3 and 5 CuO. SO^3 are soluble only 1 part in 40,000 and 1 part in 1,000,000, respectively, according to Pickering (4). It would seem that such a precipitate of bordeaux mixture, because of its insolubility and toxicity, should be more nearly ideal than soluble disinfectants for applying to the union of the grafts. The toxic action of copper sulfate on apple grafs has already been discussed. Its action on fungus spores which are somewhat comparable to abcteria is also well known.

THE RESULTS OBTAINED BY DIPPING APPLE GRAFTS IN A THICK BORDEAUX MIXTURE PRECIPITATE

Laboratory, greenhouse and short-time field trials showed the possibility of using bordeaux mixture on apple grafts. These preliminary trials indicated that a strong bordeaux mixture caused much less injury than the soluble disinfectants. The extent and possible effect of this precipitate on preventing crown gall infection is not known.

Trials with a strong bordeaux mixture on grafts wrapped with varying amounts of string are reported in table IX. Where the

TABLE IX—THE EFFECT OF DIPPING APPLE GRAFTS IN A THICK BORDEAUX MIXTURE PRECIPITATE

No. Exp.	Variety	Treatment	Fungicide	No. grafts set	Per-centage stand	Growth	Per-centage galled
1	Gano	Extra string wrap	B.M. (25-25-50) 5 seconds	49	85.7	Very good	0.0
2	Gano	String and cloth	B.M. (25-25-50) 5 seconds	49	91.8	Very good	0.0
3	Gano	Calico over string	B.M. (25-25-50) 5 seconds	51	88.2	Very good	91.0
4	Gano	Calico over string	B.M. (25-25-50) 5 seconds	51	54.5	Good	22.2
5	Gano	Cloth, no string	B.M. (25-25-50) 5 seconds	45	91.1	Good	20.0
6	Gano	Orig. string	Check	58	82.7	Very good	92.7
7	Wealthy	Orig. string	B.M. (25-25-50) 30 seconds	45	48.8	Poor	85.7
12	Wealthy	Orig. string	Check	50	68.0	Good	83.3
13	Ben Davis	Calico, no string	B.M. (25-25-50) 5 seconds	48	87.5	Good	81.8
22	Wolf River	*	B.M. (25-25-50) 5	51	1.9	Medium	20.0
26	Wolf River	New grafts	Check	48	85.4	Good	77.7

*Grafts calico wrap, base of stock dipped in white lead plus mercuric chloride.

string was applied over the original wrap and then dipped in bordeaux mixture (25-25-50), no crown gall developed. Where cloth was wrapped about the union over the string and not treated, experiment No. 3, 91 percent of the trees showed crown gall. Bordeaux mixture (25-25-50) applied on ordinary string-wrapped grafts, experiment No. 4, gave 22 percent crown gall, while the check experiment, No. 6, showed 92.7 percent.

Increasing the amount of string on the union and dipping the grafts in bordeaux mixture (25-25-50) for five minutes, did not reduce the stand, but eliminated crown gall entirely, as shown in experiment No. 1.

As shown above, a heavy wrap of string or cloth with bordeaux mixture (25-25-50) gave very good results as far as the stand was concerned. Where calico was applied over the string and dipped in bordeaux mixture, the stand was fully normal and crown gall was eliminated. On the other hand, the check experiment No. 3 showed 91 percent galled. In experiment No. 7, where a strong bordeaux mixture (25-25-50) was used on the original string wrap, and in No. 13, where the string was replaced by calico, the stand was respectively 48.8 and 87.5 percent. The percentage of crown gall was 85.7 and 81.8, respectively.

In 1917 more extensive field experiments were undertaken, using bordeaux mixture alone and also in combination with various substances to increase the adhesiveness of the mixture. Only one variety was used in these studies, as shown in table X, namely: Patten Greening. The 25-25-50 bordeaux mixture, besides reducing the percentage of crown gall, very materially reduced the stand, as shown in experiment 0-3, where the percentage of gall is 4.7 and the stand 37.5. The check on this treatment shows 30.7 and 68.5 percent, respectively. On the other hand, grafts treated with a 4-4-50 bordeaux mixture, shown in experiment 0-7, gave 24.7 percent crown gall, and in 0-8, 48.1 percent. In the 0-8 experiment the stand was reduced to 22 percent and the amount of gall was high. The increase in the amount of gall may have been induced in part by the toxic action of the resin sticker, which was added to the bordeaux mixture on the grafts.

The most important fact brought out in table X is the favorable action of the 8-8-50 bordeaux mixture. It did not reduce the stand, while it did reduce the amount of crown gall. This is quite apparent in experiments 0-11, 0-12, 0-13 and 0-14, where bordeaux mixture (8-8-50) was used alone, and in combination with lead arsenate, soap and flour. The stand in these four trials ranged from 52.4 to 72.5 percent, as compared with 68.5 percent in the check, experiment 0-15. The crown gall in these same trials varied from 9.6 to 13 percent, while the check showed 30.7 percent. The resin sticker was injurious to the stand when

TABLE X—THE INFLUENCE OF BORDEAUX MIXTURE ON THE DEVELOPMENT OF CROWN GALL

No. Exp.	Variety	Treatments	No. grafts set	Percentage stand		Percentage galled
				1917	1919	1919
O-3	Patten	B. M. (25-25-50	200	48.0	37.5	4.7
O-4	Patten	B. M. (25-25-50)+resin sticker	200	42.5	38.0	6.6
O-5	Patten	B. M. (25-25-50)+soap 10 lbs.	200	22.5	21.0	0.0
O-6	Patten	B. M. (25-25-50)+lead arsenate	200	57.0	53.0	6.1
O-7	Patten	B. M. (4-4-50)	200	64.0	60.0	24.7
O-8	Patten	B. M. (4-4-50)+resin sticker	200	40.5	22.0	48.1
O-9	Patten	B. M. (4-4-50)+soap 10 lbs.	200	67.0	64.0	13.4
O-10	Patten	B. M. (4-4-50)+3 lbs. lead arsenate	200	75.5	71.0	15.3
O-11	Patten	B. M. (8-8-50)	200	58.5	58.5	13.0
O-12	Patten	B. M. (8-8-50)+soap 10 lbs.	200	57.5	52.5	9.8
O-13	Patten	B. M. (8-8-50)+lead arsenate	200	75.0	72.5	12.4
O-14	Patten	B. M. (8-8-50)+35 lbs. flour	200	63.5	56.0	9.6
O-15	Patten	Check	200	80.0	68.5	30.7

used in both the weak and the strong bordeaux mixtures. It is interesting to note that lead arsenate, soap and flour did not affect the stand and may have increased the value of the bordeaux mixture as a preventive.

THE EFFECT OF BORDEAUX MIXTURE ON THE STAND IN THE FIELD

In 1918 the Mount Arbor Nurseries, of Shenandoah, Iowa, treated 200,000 grafts with bordeaux mixture 16-16-50. At the same time certain other lots of trees were treated with different strengths of bordeaux mixture, ranging from 4-4-50 to 25-25-50. It was not possible to hold checks with every variety, due to the size of the lots, but checks were held in the varieties where several different strengths of bordeaux were tried.

In the fall of the same year the different lots receiving different treatments were counted and the stand calculated. The data secured are tabulated in table XI. The stand in the checks of the Jonathan, Oldenburg (Duchess) and Yellow Transparent varieties was 40.3, 43.7 and 27.9 percent, respectively. In the first named variety the strong bordeaux mixture, 25-25-50, gave a stand of 30.5; the 16-16-50 mixture, 43.3; and the 8-8-50 mixture, 48.2 percent. In the Oldenburg (Duchess), the strong bordeaux mixture gave a stand of 40.9; the 8-8-50, 40.8; and the 4-4-50, 41 percent. In Yellow Transparent there was a differ-

TABLE XI—THE EFFECT OF DIFFERENT STRENGTHS OF BORDEAUX MIXTURE ON THE STAND OF APPLE GRAFTS UNDER FIELD CONDITIONS

Variety	Treatments	No. grafts planted 1919	Trees growing 1920	Percentage stand 1920
Jonathan	Bordeaux mixture 8-8-50	5964	2883	48.2
Jonathan	16-16-50	4676	1944	43.3
Jonathan	25-25-50	1668	523	30.5
Jonathan	Check	4646	1334	40.3
Oldenburg (Duchess)	8-8-50	10532	6495	40.8
Oldenburg (Duchess)	Check	3000	1311	43.7
Oldenburg (Duchess)	25-25-50	2689	1100	40.9
Oldenburg (Duchess)	4-4-50	3000	1231	41.0
Yellow Transparent	8-8-50	6492	2095	32.2
Yellow Transparent	Check	994	274	27.9
Whitney	8-8-50	1800	768	37.6
Whitney	25-25-50	480	136	28.3

ence of about 4 percent in favor of the treated trees. In the case of two lots of Whitney treated, one with 25-25-50 and another with 8-8-50, the effect of strong bordeaux mixture is again obvious, the stand is 28.3 and 37.6 percent, respectively.

It would seem that the use of bordeaux mixture under ordinary field conditions does not seriously reduce the stand, even when used, as strong as 16-16-50 and 25-25-50. The weaker mixtures, such as 8-8-50 and 4-4-50, probably induce no injury, altho the data presented in table XI show little difference in the effect of weak and strong mixtures.

It must be recognized that in using percentage of stand as an indicator of possible injury due to the bordeaux precipitate on the union, many variable conditions enter. In the first place, the grafts may not all be made from the same lot of scion wood, and different men may have made the grafts. Some men make better grafts than others, which leads to a better stand. Again, some grafts are made in January and others in March, while they are all set at about the same time in the spring. In the field it has been repeatedly observed that some men are more careful in planting than others. In other cases the grafts may be injured, or killed by careless drivers on the packing machine. When consideration is given to these sources of variation, it is obvious that percentage of stand is not always a safe indication, even tho quite a large number of grafts are used. (About 20,000 are required to set one acre.) It is only by collecting data over a series of years that reliable information can be obtained. However, it should be emphasized that the trials that have been made are sufficient to indicate clearly the feasibility of dipping apple grafts in bordeaux mixture.

CONCLUSIONS

The following tentative conclusions seem justifiable from the experiments recorded.

The infection of apple grafts is readily accomplished by dipping the grafts just before planting in a viable bouillon culture of *Bacterium tumefaciens*. The majority of the galls occur at the union. The stock is less liable to become infected than the scion.

Apple grafts were apparently equally susceptible to the crown gall organism where the callous was normal, excessive or slight.

Well made and poorly made grafts showed little difference in the amount of crown gall that developed.

Using an unusually large amount of string over the union of the grafts leads to girdling and excessive callousing of the trees, which seems to facilitate crown gall infection. Cloth applied over the union as a wrapper, either with or without string, decreases the amount of crown gall.

Scion wood cut from trees infected with crown gall at the union did not show any increased amount of crown gall.

Hairy root seedlings, when used as stock, did not transmit hairy root to the scion, but the stock portion of the graft remained infected in the majority of cases.

Most of the crown gall infection takes place the first year on the grafts, during the formation of the callous, at the union.

Surface disinfection with formaldehyde (.16 percent), copper sulfate (.25 percent) and mercuric chloride (.1 percent), were seriously injurious to callousing of apple grafts. Fungicides which go into solution slowly, such as lead arsenate and bordeaux mixture, have a much less injurious effect on the callousing process.

A strong bordeaux mixture (25-25-50) decreases the stand, tends to have a preserving action on the string and reduces the amount of crown gall.

Resin sticker added to bordeaux mixture increases its toxic action and reduces the stand. The addition of lead arsenate or soaps to bordeaux mixture does not increase its toxic action on the grafts, but rather increases its adhesiveness and its fungicidal efficiency.

More dilute bordeaux mixtures did not reduce the stand and proved nearly as beneficial in reducing crown gall as the stronger mixtures. The use of bordeaux mixture (8-8-50), with or without lead arsenate, reduced the percentage of crown gall about 66 percent over the checks, and nearly 50 percent over the mean percent of crown gall in all the checks in the Wealthy variety.

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